Neuroscience Research 74 (2012) 210–215

Contents lists available at SciVerse ScienceDirect

Neuroscience Research

journal homepage: www.elsevier.com/locate/neures

Effects of age-related dopaminergic neuron loss in the substantia nigra on the circadian rhythms of locomotor activity in mice

Makoto Tanaka[a], Eriko Yamaguchi[a], Mami Takahashi[a], Kana Hashimura[a], Takao Shibata[a], Wataru Nakamura[b], Takahiro J. Nakamura[a,*]

[a] Faculty of Pharmaceutical Sciences, Teikyo Heisei University, 4-1 Uruido-Minami, Ichihara, Chiba 290-0193, Japan
[b] Laboratory of Oral Chronobiology, Graduate School of Dentistry, Osaka University, Suita, Osaka 565-0871, Japan

ARTICLE INFO

Article history:
Received 2 May 2012
Received in revised form 28 August 2012
Accepted 26 September 2012
Available online 5 October 2012

Keywords:
Circadian rhythm
Dopaminergic neuron
Substantia nigra
Tail suspension test

ABSTRACT

Elderly people often develop sleep and autonomic dysfunctions, which are regulated by circadian rhythm. Recently, we reported on the degradation of neural output from the central circadian clock in the suprachiasmatic nucleus (SCN) with aging. However, it is likely that many other factors contribute to the age-related decline in the functioning of the circadian system. In this study, we examined the effects of dopaminergic neuronal loss in the substantia nigra (SN) on circadian rhythms of mice to assess whether age-related degeneration of the dopamine system influences circadian rhythm. Young male C57BL/6J mice were administered 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a compound that selectively destroys dopaminergic neurons in the SN, and their wheel-running activities were recorded. We observed that MPTP-treated mice lost 43% of their dopaminergic neurons in the SN (on average) and demonstrated longer period of wheel-running activity rhythm in constant darkness compared with control mice. However, all the remaining circadian parameters in the MPTP-treated mice remained constant. Our findings suggest that in addition to SCN output dysfunction, age-related degeneration in the dopamine system of the brain leads to circadian rhythm irregularities.

1. Introduction

In humans, alterations in sleeping patterns, including duration and quality, and specific sleep pathologies and disorders, are associated with aging. Aging not only impacts the circadian rhythm of humans but also animals. For example, in aged mollusks (*Aplysia* spp.), the circadian rhythm in optic nerve impulse frequency, recorded either *in vivo* or *in vitro*, is reduced in amplitude and regularity (Sloan et al., 1999). In rats and hamsters, aging is also associated with a decreased amplitude of locomotor activity rhythms (Penev et al., 1997; Scarbrough et al., 1997), as well as the increased fragmentation of activity rhythm (Turek et al., 1995), a shortened or lengthened free-running period (Pittendrigh and Daan, 1974; Davis and Viswanathan, 1998), a slowed rate of re-entrainment following a shift in the light–dark (LD) cycle (Zhang et al., 1996), and an altered sensitivity to light (Valentinuzzi et al., 1997). In mice, in addition to the degradation of the amplitude and regularity of the locomotor rhythm, aging has a prominent effect on re-entrainment following LD cycle changes (Valentinuzzi et al., 1997). In humans, the most reproducible effect of age on circadian behavior is the deterioration of rest/activity rhythm, resulting in a sharp decrease in quality of life (Witting et al., 1990). The proportion of the population comprising elderly people in increasing; therefore, it is very important to understand the mechanisms of age-related sleep disorders. Sleep disruption significantly impacts the quality of life of the aged population and their caregivers (Bliwise, 1993; Dijk et al., 1999; Turek et al., 1995; Van Someren, 2000).

The wake–sleep cycle is regulated by the circadian clock, which is located in the suprachiasmatic nucleus (SCN) of the anterior hypothalamus. The rhythms are generated by a cell-autonomous circadian oscillator, which is synchronized with the external environment by light through the retinohypothalamic tract; hence, the behavior of the organism within the daily 24-h LD cycle is synchronized by the presence of light (Moore, 2007). A study of *in vivo* electrical activity of SCN neurons recorded from freely moving mice revealed that profound changes occurred in circadian electrical rhythm in middle-aged mice compared with younger animals (Nakamura et al., 2011). This result suggests that aging degrades neural output from the SCN. Although many other studies also supported this evidence (Krajnak et al., 1998; Kawakami et al., 1997; Wise et al., 1988; Sutin et al., 1993; Zhang et al., 1996; Satinoff et al., 1993; Watanabe et al., 1995; Aujard et al., 2001), there are likely to be many other factors that contribute to age-related declines in the circadian system.

* Corresponding author. Tel.: +81 436 74 6159; fax: +81 436 74 6159.
E-mail address: t.nakamura@thu.ac.jp (T.J. Nakamura).

0168-0102/$ – see front matter
http://dx.doi.org/10.1016/j.neures.2012.09.005

Previous studies have demonstrated that elderly people show a steady decline in the number of dopaminergic neurons in the substantia nigra (SN) of the midbrain, and almost 40% fewer dopaminergic neurons are left in this area by the age of 60 years (Rehman and Masson, 2001; McGeer and McGeer, 1976; Mann et al., 1984). This cell loss is accompanied by a 50% reduction in dopamine content, tyrosine hydroxylase activity, and radioligand binding to catecholamine synaptic vesicles in the neostriatum (Adolfsson et al., 1979; Carlsson and Winblad, 1976; McGeer and McGeer, 1976; Allard and Marcusson, 1989).

A neurotoxin precursor, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), causes the direct degeneration of dopaminergic neurons in the SN, either by necrosis or apoptosis (Przedborski et al., 1996). Therefore, this toxin is widely used in *in vivo* animal models for Parkinson's disease (PD), such as rodents and nonhuman primates. In young mice, injections of MPTP (30 mg/kg intraperitoneally) 5 times daily have been shown to reduce the levels of dopamine in the neostriatum by approximately 60% and to cause the death of dopaminergic neurons in the SN (Heikkila et al., 1984). Thus, the administration of MPTP in animal models is not only useful for studies on PD but also for studies on the elderly population in terms of dopamine system degeneration in the brain.

In this study, to assess that age-related degeneration of the dopamine system influences circadian rhythmicity, we examined whether the depletion of dopaminergic neurons in the SN affects the circadian behavioral rhythms of mice. We monitored the diurnal and circadian rhythms of wheel-running activity in mice treated with MPTP. After these had been recorded, a tail suspension test (TST) was conducted to deduce the level of psychological deficiency in the mouse, and the brain was stained with antibodies targeting tyrosine hydroxylase (TH) to assess the number of dopaminergic neurons in the SN.

2. Materials and methods

2.1. Animals

Twenty 8-week-old male C57BL/6J mice were purchased from Charles River (Yokohama, Japan) for this experiment. Animals were kept in a laboratory with regulated air conditions (room temperature: $23 \pm 1\,^{\circ}C$; humidity: $50 \pm 10\%$) and a controlled LD cycle (12 h of light and 12 h of darkness, with a light intensity of 200–300 lx) until the beginning of the experiment. Food and water were available *ad libitum*. All procedures and standards of care were approved by the Institutional Animal Care and Use Committee of the Teikyo Heisei University (approval ID#: 2010-008) and were conducted according to the guidelines of the Japanese Physiological Society for the use of experimental animals.

2.2. Recording locomotor activity and MPTP administration

The C57BL/6J mice were divided in to 2 groups: control group ($n = 10$) and MPTP-treated group ($n = 10$). Each mouse was transferred to a 150-mm (width) × 300-mm (length) × 150-mm (height) cage that contained a running wheel (100 mm in diameter) in a light-tight photoperiod box (equipped with a fan and a timer-controlled light) with a 12 h/12 h LD cycle (light-on time 06:00 am). Locomotor activity was measured as running-wheel revolutions recorded in 1-min bins using The Chronobiology Kit (Stanford Software Systems, Santa Cruz, CA, USA). MPTP hydrochloride (Sigma–Aldrich Japan, Tokyo, Japan) was dissolved in saline and administrated to the mice intraperitoneally at zeitgeber time (ZT) 7–8 (ZT 12 is defined as the lights-off time) for 5 days at a dose of 30 mg/kg of bodyweight. Control mice were administered with saline alone.

2.3. Tail suspension test

The TST procedure was first described in a previous study by Steru et al. (1985). In our test, an elastic band (3 cm in diameter) was used to suspend the mice by their tails. Adhesive tape was used to attach the tail to the band (at ~1 cm from the tip of the tail), which was hooked onto a horizontal rod. The distance between the tip of the mouse's nose and the floor was approximately 20 cm. The mice were suspended for 7 min, and the time spent immobile during the last 5 min was measured for each animal by at least 2 observers who were blinded to the treatment. The results were then averaged.

2.4. Immunohistochemistry

The methods of immunohistochemistry (IHC) were similar to those described previously by Nakamura et al. (2011). In brief, mice were deeply anesthetized with ether and perfused with phosphate-buffered saline (PBS), followed by 4% (w/v) paraformaldehyde in PBS. Their brains were dissected, postfixed overnight at 4 °C, and cryoprotected in 30% sucrose in PBS. IHC was performed on free-floating 30-μm cryostat coronal brain sections. Sections were washed for 5 min with PBS (3 times), and were then incubated for 10 min in 2% H_2O_2 in PBS. Sections were washed again in PBS (3 times), dipped in 10% normal goat serum in PBS for 1 h, and incubated overnight with a 1:2000 dilution of antibodies targeting TH (Santa Cruz biotechnology, Santa Cruz, CA, USA) in PBS at 4 °C. The next day, the sections were washed in PBS again (3 times) and incubated for 2 h with biotinylated goat anti-rabbit antibodies (dilution: 1:200). Again, sections were washed for 5 min in PBS (3 times), dipped in AB solution (Vector Laboratories, Burlingame, CA, USA) for 45 min, and washed again in PBS before they were placed in filtered 0.05% 3,3′-diaminobenzidine in PBS containing a 1:10,000 dilution of 30% H_2O_2. After a 2–3-min duration for sufficient color reaction, the sections were washed with PBS and immediately mounted onto slides. The sections were dried overnight, washed with water for 10 min, dehydrated with ethanol and protected with a cover slip. ELIPSE Ni camera systems (Nikon Instruments Inc., Tokyo, Japan) were used to capture images of the brain section. All immunopositive cells within the SN pars compacta (SNc) region were counted manually with the aid of a grid at a magnification of 100×. All immunopositive cells were counted, regardless of the intensity of their staining. Two observers who were blinded to the treatment performed the counts, and the results were then averaged. No positive staining was observed in the control experiments, which did not include the primary antibody.

2.5. Data analysis and statistics

The wheel-running data were analyzed using ClockLab software (Actimetrics, Wilmette, IL, USA), and data on the activity of the mice were double-plotted on actograms using a 1-min block size. Chi-square periodogram analyses for 2 weeks of continuous data were performed to calculate circadian rhythmicity. The duration of each cycle that was devoted to running wheel activity was designated alpha (α), and the duration of non-wheel-running activity was designated rho (ρ). To assess the parameters, the average pattern of activity (*i.e.*, activity profile) was determined at modulo-period for each animal in complete darkness (DD) for 14 cycles. For each waveform, α was calculated, which was the duration in which motor activity was greater than the medium. Fragmentation was calculated from the number of activity bouts (maximum gap: 21 min; threshold: 3 counts/min) per day. The statistically significant differences between the 2 groups were determined using a Student's